Figure Supplementary 1: TEM1 heterologous prime/boost vaccination increases antitumor effect. (A and B) $2 \times 10^5$ splenocytes from BALB/c mice vaccinated with the plasmid + Ad5 scheme were co-cultured with each individual peptide contained in pool A (Facciponte et al., 2014) (A) and with the 15-mer (TEM1$_{31-45}$) and the 12-mer (TEM1$_{34-45}$) peptides (B) in ELISpot assay. Bar charts illustrate number of IFN-γ spots. Means ± SEM are shown from 1 experiment with 4 mice/group.
Figure Supplementary 2: TEM1 heterologous prime/boost vaccination increases antitumor effect. BALB/c mice were subcutaneously injected with CT26 cells in the lower back and immunization initiated 5 days after tumor inoculation, repeated at weekly intervals for three weeks. Tumor growth was monitored throughout the experiment. Tumor growth for each individual mouse is shown for one representative experiment out of 3.
Figure Supplementary 3: Heterologous TEM1 vaccine and early RT results in augmented anti-tumor effects. BALB/c (A) and C57BL/6 (B) mice were subcutaneously injected with CT26 or TC1 cells in the lower back, respectively. Immunization was given 3-5 days after tumor challenge and continued at weekly intervals (±2 days). Tumor irradiation (21 Gy single dose, (Uribe-Herranz et al., 2020)) was performed at day 12 in CT26 (200-350mm³ tumor volume) or at day 10 in TC1 (100-250mm³ tumor volume) after tumor implantation (early RT) or alternatively on day 21 in CT26 (1600 mm³ mean of tumor volume) or day 19 in TC1 (1450 mm³ mean of tumor volume) (late RT). Tumor growth was monitored throughout the experiment. Tumor growth for each individual mouse is shown for one representative experiment out of 3.
Figure supplementary 4: Dual treatment with heterologous TEM1 vaccination and RT reduces abscopal tumor growth. BALB/c (A) and C57BL/6 (B) mice were injected with CT26 or TC1 cells, respectively. Tumors were injected in both flanks of each mouse at 2-day interval, and then RT was performed only in the irradiated tumor according to the early RT schedule (Plasmid-RT-Ad5-Ad5). Growth of both primary and abscopal tumors were followed. Graphs showing abscopal tumor growth of individual mice are demonstrated. Data of one independent experiment out of 3 are shown.
Figure Supplementary 5: Dual treatment promotes MHCI expression and immune recognition of endothelial cells by anti-TEM1 T cells enhancing tumor-associated vasculature injury. TC1-bearing mice treated with various therapies were sacrificed to assess their immune response in the peripheral blood. (A) ELISpot assay performed on splenocytes stimulated with a TEM1 peptide library (A) depicts the frequency of peripheral peptide-specific immune responses. (B) CT26-bearing mice that received local RT were sacrificed to harvest spleen. ELISpot assay was performed on the splenocytes of mice after stimulation with TEM1 library pool A and pool C. CT26-bearing mice were sacrificed 1 day after RT to harvest tumors. (C) Flow cytometry analysis performed on primary tumor shows MHCI levels on the surface of CD45+CD31+ endothelial cells. 2-tailed t test analyses were performed (NO RT vs RT P=0.046). (B) RT-PCR shows fold-change of Tem1 mRNA. Tukey's multiple comparison tests were performed (CTRL vs RT P=0.002; TEM1 vs RT P=0.004; CTRL or TEM1 vs TEM1 + RT P<0.001). All measurements were performed in triplicate. Means ± SEM are shown from 1 representative experiment out of 3. *P < 0.05, **P < 0.01, ***P < 0.001, N.s. Non significant.
Figure Supplementary 6: Dual treatment enhances DC/macrophage activation, promotes TAA cross-priming and boost T cells infiltration. TC1-bearing mice treated with various therapies were sacrificed to assess their immune response in blood and tumor. (A and B) ELISpot assay performed on splenocytes stimulated with a TEM1 peptide library (B) or E7 peptide (A and B) depicts the frequency of peripheral peptide-specific immune responses. (C) In vitro growing CT26 cells were exposed to different doses of radiation and expression of markers were analyzed 24h later by flow cytometry. One-way ANOVA was performed (P=0.003). (D) CT26-bearing mice were sacrificed and IHC was performed on abscopal tumors to quantify CD3+T cell infiltration. Representative CD3+ staining images (20X and 80X). Mean of 5–6 fields per mouse and 3–4 mice per group were analyzed (CTRL vs TEM1 + RT P<0.0001; TEM1 vs TEM1 + RT P=0.016; RT vs TEM1 + RT P =0.0003). Means ± SEM are shown from 1 representative experiment out of 3. (E and F) TC1-bearing mice treated with various therapies were sacrificed to collect their tumors. Flow cytometry analysis shows percentage of E7-specific T cells (E7-Tet.) in collagenase-digested tumor cell suspensions in the abscopal (E) and primary (F) tumors. Means ± SEM are shown. *P < 0.05, **P < 0.01, ***P < 0.001, N.s. Non significant
Figure supplementary 7: Tumor, splenocyte and lymph nodes single-cell suspensions were analyzed using polychromatic flow cytometry and progressive gating strategy. Representative staining with LIVE/DEAD, CD45, CD11b, CD11c, CD40 and MHC-II is used to identify the DC/Macrophage population in tissues (Lymph nodes) of treated mice. Representative staining with LIVE/DEAD, CD45, CD3, CD4, CD8, gp70-tetramer and PD1 is shown to quantify helper (CD4+), cytotoxic (CD8+), gp70-specific (tetramer) and PD-1+ T cells in tumor suspension.
Figure supplementary 8: Inhibition of PD-1/PD-L1 axis enhances the anti-tumor effects of dual therapy. In vitro growing CT26 (A) and H5V (B) cells were exposed to increasing doses of ionizing radiation and surface levels of PD-L1 were analyzed 24h later by flow cytometry. (C) CT26-bearing mice were sacrificed and their tumors were analyzed. RT-PCR shows fold-change quantification of IFN-γ expression levels. All measures were made in triplicates. Means ± SEM are shown. Each dot represents a mouse.
Figure Supplementary 9: C57BL6 mice were injected subcutaneously with TC1 tumor on the lower back at day 0, heterologous TEM1 vaccination began at day 3, and localized RT followed at day 10 after tumor inoculation. Concurrent anti-PD-L1 blockade was administered three times, at 3-day intervals, beginning 10 days after tumor injection. (A) Individual mouse tumor sizes are shown. Differences in tumor volume were evaluated with two-way ANOVA test (TEM1 + RT vs TEM1 + RT + PD-L1 P=n.s.). (B) The Kaplan-Meier plots depict probability of survival of mice in each group. Statistical significance was calculated with Log-rank (Mantel-Cox) test. (C) 1 x 10^6 splenocytes were stimulated overnight with the E7 peptide and assayed for IFN-γ by ELISpot. Data is representative of one independent experiment out of two.
Figure supplementary 10: Inhibition of PD-1/PD-L1 axis with heterologous TEM1 vaccination and RT augments gp70-specific responses while reducing the TEM1-specific response. (A) CT26-bearing mice were sacrificed at day 24 after abscopal tumor inoculation and their spleens analyzed by flow cytometry to estimate frequency of gp70-specific T cells after each treatment. Means ± SEM are shown. Each dot represents a mouse. (B) Tumor-free BALB/c mice were vaccinated with either 3 injections of TEM1 vaccine or gp70 vaccine and IFN-γ ICS was performed on blood to assess vaccination efficacy. Flow cytometry analysis shows frequency of circulating vaccine-specific T cells (IFN-γ+CD8+) after overnight stimulation with TEM1 peptide pool or gp70 peptide. Means ± SEM are shown. Each dot represents a mouse.
Figure supplementary 11: Inhibition of PD-1/PD-L1 axis with heterologous TEM1 vaccination and RT augments gp70-specific responses while reducing the TEM1-specific response. CT26 tumor-bearing mice were adoptively transferred (ACT) with TEM1- or gp70-specific T cells isolated from vaccinated mice. In some groups, mice were also treated with RT at day 12 (one day before ACT). Tumor growth from individual mice is shown. Data is representative of 1 independent experiment out of two.
Supplementary Figure 12: Our model shows that administering radiation in combination with TEM1 vaccination boosts the TEM1-specific T cell response. Radiation augments MHCI expression on endothelial cells, enhances their immune recognition by anti-TEM1 T cells and results in increased endothelial and tumor cell death. Dead cells are subsequently scavenged by RT-activated DC/macrophage that process and cross-present additional TAA to T cells. This secondary cross-priming event (i.e., epitope spreading) promotes expansion of TAA-specific T cells, that work in concert with the anti-TEM1 immune response to eradicate larger numbers of tumor and endothelial cells, resulting in improved control of primary and abscopal tumors. Addition of anti-PD-L1 to the dual therapy enhances the gp70-specific response and improves the anti-tumor effect.